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ON THE SPOROZOON PARASITES OF THE FISHES OF WOODS HOLE AND VICINITY

I. FURTHER OBSERVATIONS ON MYXOBOLUS MUSCULI FROM FUNDULUS

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My knowledge of several points relating to the life history, structure, and habits of *M. musculi* as described in a previous paper was incomplete. More recent studies have supplied interesting additions to and confirmation of previous observations. The new matter relates to the method of infection, the pathological effects, the mode of attack, the distribution of the disease within the species, and certain obscure stages of the life cycle.

DISTRIBUTION OF THE PARASITE IN NATURE

Hitherto my observations have been made upon fish that had been captive for one or more days. Since a very large proportion of them were found to be infected with both bacteria and Myxosporidia, there seemed to be good grounds for expecting fish at large to be infected in rather large numbers. But this does not seem to be the case. When Fundulus are carefully examined immediately after reaching the laboratory, the number of fish having lesions of any kind are surprisingly few. In one catch of one thousand fish, only eleven had pathological abnormalities. One of these eleven fish was infected with *Myxobolus musculi*. From another catch of one hundred and seventy-five fish, Myxosporidia were found only in three fish which had lesions in the integument, there being no other fish having injuries. In a third catch of sixty-five fish the integument had typical lesions containing the parasites in but two cases. All of these counts were made when the water was at a temperature lower than the maximum in the vicinity of Woods Hole. The earliest count made was in July of a remarkably cold season. The proportion having Myxobolus was 4.4 per cent, while those taken in the latter part of the month of August had as low as 0.1 per cent infected.

The temperature is no doubt an important factor. The water of the indoor aquaria is warmer than that of the ponds and bays where

the fish are commonly caught. This, in part, accounts for the larger percentage of infection in fish that have been confined a day or two. But there are two other factors. It has already been demonstrated (Hahn, 1913: 193) that injuries to the integument encourage the entrance of the Myxobolus. An examination of the gills of a number of Fundulus has recently revealed the fact that *M. musculi* is far more common on the gills of fish that are apparently healthy than it is in the integument and muscle. Fish having injuries and confined in aquaria are therefore exposed to infection from the gills of a comparatively large number of previously infected fish. These facts explain the discrepancy in the distribution of the parasites as found in captive fish and in free fish.

EXPERIMENTAL TRANSMISSION OF THE M. MUSCULI AND THE CONDITIONS FAVORABLE FOR RECOVERY

In order to confirm the results of previous experiments along this line, two experiments were undertaken. Twelve Fundulus were placed in one aquarium jar having a capacity of at least 5 gallons and supplied with running water. Six fish were put into a second jar for the purpose of a control. The six controls had incisions cut in the integument in exactly the same manner as the fish which were inoculated, but a sterile scalpel was used. Bits of tissue known to contain the myxospores of *M. musculi* were inserted into pockets made with a clean scalpel under the scales of the opercle and head of six of the twelve fish above mentioned. Similar bits of tissue were inserted into incisions made in the integument of the remaining six fish so as to be in contact with the body muscle.

By the second day after the operation, all of the eighteen fish were still active. The wounds of all had developed into open infected sores, due, no doubt, to the bacteria which enter from the water. But there was far greater activity in the wounds of the twelve fish which had received infected tissue. The adjacent integument was rough, swollen and the scales were loosened. In some the flesh was exposed for a distance around the incision and a thick layer of white flaky flesh was about ready to fall out of the wound. This condition is unmistakably due to the destructive work of the Myxosporidia. Those fish which had received infection in the head region had more or less inflammation in the vicinity of the lesion and in some cases it had spread under the jaw and to the opposite side of the head. In one case the roof and floor of the mouth were found later to be highly infected with Myxobolus. This fish and one of the controls died on the second day of the experiment. The latter had a bad wound which proved to have numerous myxospores. They probably entered the wound from the

water or found their way in some way from the gills of one of the controls. As stated before, recent observations have shown that *M. musculi* is rather common in the gills of fish that show no signs of disease. The same conditions apply to a second control which died on the third day. The other four controls recovered and lived throughout the period of observation.

By the sixth day five of the inoculated fish died from the effects of the Myxobolus. The parasite was found in the infected tissues in each case. Altogether, eight of these fish died, three escaped, and after twenty-three days the remaining fish had apparently recovered. The three that escaped were seriously afflicted when last seen.

This experiment was repeated with some slight modifications for the purpose of gaining more light upon the natural immunity of the host. Infected material was introduced under the integument of four Fundulus as follows: (1) Fragments of tissue containing myxospores were placed under the integument of the operculum; (2) the same material was introduced under the integument of another fish on the dorsal side just between the eyes; (3) infected material was pushed into slits cut into the integument around the mouth; (4) the infected tissue was introduced into the flesh on the left side of the body. These four fish were given plenty of food and fresh water. They had been confined for thirteen days so that it was safe to assume that there were no well developed infections at the beginning of the experiment. No controls were kept.

The locus of the infections all developed into conspicuous lesions. The fourth fish developed a large open sore, three-fourths of an inch in diameter, with white opaque flesh. It died on the sixth day. The muscle around the area over which the integument remained unbroken was rich in the trophic stages of the Myxobolus, including some propagative stages. In the tissue used to infect this fish there were few, if any trophoblasts of either propagative or multiplicative stages. Myxospores were very abundant and other propagative stages were probably present. It seems likely that the new host was infected by the latter. The rapid hypertrophy of the tissues is characteristic of the disease and tends to show that the fish has little or no defence when muscle tissue is attacked.

In Fish No. 1 the muscle of the back and sides was involved by some means, probably by the spread of the disease to the dorsal side of the operculum. Here again a typical lesion was developed and resulted fatally.

The fate of the other two fish was very different. After twenty-six days both were alive and their wounds were healing rapidly. At first, both these fishes appeared to have wounds sufficiently serious to cause their death. But the thin subdermal connective tissue over the skull

either does not conduct the parasites beyond the reach of immunizing agents as in the case of the body muscle, or saprophytic bacteria and their toxins have not the favorable conditions to poison the host that are provided when the infection occurs in body muscle. Inasmuch as there is ample evidence that *M. musculi* does attack epidermis and connective tissue, one must conclude that in this case either the defense of the fish was sufficient to destroy the parasite before it spread to the body muscle or that the parasite passed through its trophic stages and had become non-virulent. In the fish which received infection through the muscles of the lower jaw, there was nothing to limit the spread of the virulent stages into muscles where it would be fatal, such as the eye muscles. One is therefore inclined to the view that the parasites pass into a comparatively inactive condition. This would require a very simple explanation, namely, that the trophic stages develop simultaneously into sporogenic stages. Such was doubtless the case with most of the parasites in the primary host. In the latter the disease never at any time assumed very injurious conditions. Yet I have observed cases of infection in the head region which resulted fatally. This particular fish lived for over a month after the disease was first observed on the middle of the opercle. It did not spread beyond the border of the opercle, and when last observed at the end of the season the wasted tissues were rapidly regenerating. At the start, myxospore and sporoblast stages alone were encountered in large numbers. All of the parasites seem to have developed into sporoblasts and eventually myxospores so that the host was safe for the season unless the spores germinated again. In the two fish mentioned above, the transfer of the myxospores to another host apparently supplied the necessary stimulus, or there were still a number of trophoblasts of the propagative cycle.

The conditions of the recovery in these three cases were chiefly the location of the primary infection. Had the fish not been well fed, they would doubtless have died, as have many others having infected jaws, eyes, opercles, etc. But food alone will not explain their recovery, because I had here two and have had at other times many other fish with infections in the body muscle which nearly always kill the fish.

Recovery in the barbel when afflicted with abscesses caused by *M. pfeifferi,* is possible when there is no external lesion or when no vital organ is involved. Usually these are the conditions when the body muscle alone is infected. According to de Drouin de Bouville (1908), phagocytosis then prevents the fatal accumulation of atrophied tissue. As has been already observed, the conditions are just the contrary in the Fundulus. When *M. musculi* invades the body muscle it is rarely checked and when the attack is superficial as in the head region, the chances of recovery are good. In this conclusion I have assumed that

the myxospores of *M. musculi* are not capable of germinating in the tissues where they have matured. Mercier (1906) has established this as a frequent method of multiplication in *M. pfeifferi* of the barbel. Altogether, the evidence that the myxospores of *M. musculi* may germinate in the original host is negative. The fact that numerous myxospores were observed unaccompanied by other stages for such a long period in the case above mentioned is, in itself, a sufficient proof that, in this case at least, the necessary stimulus for germination of the myxospore was lacking.

In regard to the propagation of *M. musculi* from fish to fish, it may eventually prove that the myxospores may enter the tissues both through lesions as is indicated by the above experiments, and through the gills and through the digestive tube. Since it has been shown that *M. pfeifferi* is taken into the barbel with its food, the latter mode of infection for *M. musculi* seems the more probable, especially when it is recalled that the relations of both parasites to their host are so very similar. The attack upon the muscle fibers is almost identical in the two species.

The myxospores of *M. inequalis* which causes the disease known as carp pox, are also transmitted to new hosts by means of the food (Wierzejsky, 1898).

Contrary to my expectation, there is absolutely no evidence that Fundulus ever suffers from an internal infection by *M. musculi,* unless it be about the mouth and gill region.

In the summer of 1915 I again inoculated fish with Myxosporidia. In these experiments the ultimate object was to discover if the species of Myxobolus hitherto commonly encountered in *Fundulus heteroclitus* would grow and produce the same typical pathological conditions in *F. majalis* and *F. diaphanus,* and to see if the parasite could be recovered in the same host in one of its characteristic stages.

A *Fundulus heroclitus* which proved by examination of stained tissues to have typical large schizonts in considerable numbers was first secured. From two typical Myxobolus lesions in the lateral region of the body, bits of flesh about 1 by 3 mm. in size were removed by means of sharp sterilized forceps. The subjects were confined in clean aquaria, with running sea water for *F. majalis* and *F. heteroclitus,* and fresh water for *F. diaphanus.* They were fed regularly each day. Inasmuch as it has been shown that lesions free from Myxosporidia in fish which are well cared for rapidly recover, no controls were provided. This was partly due to the fact that one cannot be sure that the water is free from Myxosporidia, since the gills of many Fundulus may be infected and presumably disseminate the germ.

The results of operations upon thirteen fish are summarized in the following table:

TABLE 1

1	2	3	4	5	6	7	8	9
Species	Catalogue Number	Length in inches	Time of Inoculation	Time of Examination	Period of Growth	Dead or Killed	Condition of Wound (gross exam.)	Kind of Organism (based on examination of stained tissue)
Fundulus majalis	1097.1	5.5	8/24/15 12 a. m.	8/26/15 7 a. m.	19 hrs.	Died; water supply failed	Large sore on site of incision	Muscle degenerate; schizonts many in muscle
Fundulus majalis	1097.3	4.5	8/24/15 12 a. m.	8/26/15 7 a. m.	19 hrs.	Died; water supply failed	Large lesion, advanced ...	Many schizonts; muscle degenerate *
Fundulus majalis	1097.5	5.5	8/24/15 12 a. m.	8/26/15 7 a. m.	19 hrs.	Died; water supply failed	Moderate sized lesion.....	Schizonts large but few
Fundulus majalis	1097.7	3.8	8/24/15 12 a. m.	8/26/15 7 a. m.	19 hrs.	Died; water supply failed	Large lesion, advanced.....	Muscle badly degenerate; not many schizonts, probably too degenerate
Fundulus majalis	1097.13	3	8/24/15 12 a. m.	9/ 6/15	13 days	Killed	Lesions ¼"x⅛", open but shallow, white	*Slide Lost*
All except one produced serious lesions.								
Fundulus diaphanus	1097.6	4.8	8/24/15 12 a. m.	8/26/15 7 a. m.	19 hrs.	Died	Moderate lesion	Schizonts in small numbers
Fundulus diaphanus	1097.8	4	8/24/15 12 a. m.	8/26/15 7 a. m.	19 hrs.	Died	Large lesion; open wound; purulent flesh	Many schizonts and some trophoblasts
Fundulus diaphanus	1097.10	2.8	8/24/15 12 a. m.	8/26/15 7 a. m.	19 hrs.	Died from the infection	Large, with inflammation extending to pectoral fin	A few schizonts
Fundulus diaphanus	1097.11	2.8	8/24/15 12 a. m.	8/26/15 7 a. m.	19 hrs.	Died from the infection	Moderately developed lesion	Schizonts rare
Fundulus diaphanus	1097.12	3.3	8/24/15 12 a. m.	8/27/15	3 days	Died from the infection	Large area with inflammation extending over ribs	Muscle degenerate; large schizonts not numerous
Extensive lesions developed in all. Schizonts present in all but rather rare.								

* Schizonts sporulating.

TABLE 2

1	2	3	4	5	6	7	8	9
Kind of Fish	Catalogue Number	Length in Inches	Date of Innoculation	Date of Examination	Period of Growth	Dead or Killed	Condition of Wound	Stage of Parasite
Fundulus heteroclitus	1103.1	3	8/31/15 12 a. m.	9/1/15 10 a. m.	22 hrs.	Died, probably shock	Lesion almost unchanged...	Trophoblasts present but very rare
Fundulus heteroclitus	1103.3	4	8/31/15 12 a. m.	9/1/15 3 p. m.	27 hrs.	Died, probably from head infection	Gills diseased; head and eye infected; lesion unchanged	Myxocysts in gills; large trophoblasts few; muscle degenerate
Fundulus heteroclitus	1103.6	3.5	8/31/15 12 a. m.	9/2/15 9 a. m.	45 hrs.	Died	Badly infected about head; eye and opercles protruding; lesion not changed	Trophoblast in gills; large trophoblasts, many, in muscle
Fundulus majalis	1103.7	4.5	8/31/15 12 a. m.	9/6/15	7 days	Alive	Wound almost invisible; no evidence of disease	Schizonts few; many small trophoblasts
Fundulus majalis	1103.8	3.5	8/31/15 12 a. m.	9/6/15	7 days	Alive	Wound still open; no evidence of infection	Many trophoblasts
Cyprinodon variegatus	1103.2	1.5	8/31/15 12 a. m.	9/1/15 10 a. m.	22 hrs.	Died	Lesion not developed.....	Young trophoblasts in muscle many; muscle degenerate
Cyprinodon variegatus	1103.4	1.8	8/31/15 12 a. m.	9/1/15 3 p. m.	27 hrs.	Died	Slight infection about head; lesion but little developed	Muscle full of trophoblasts; gill has sporoblast stages
Cyprinodon variegatus	1103.5	3.5	8/31/15 12 a. m.	9/2/15	45 hrs.	Died	Inflammation on head serious; swollen eye and opercle; incision not changed much	Many large schizonts and trophoblasts; Myxocysts in gills

The right-hand column of the above table indicates the kind and number of Myxobolus in the hypertrophied tissues, especially muscle, of the operated fish. In twelve out of thirteen fish the Myxobolus was recovered after being introduced. In all cases it had multiplied and was growing in a perfectly normal way. There is no evidence that the change of host has modified the usual course of the life cycle.

Considering the last two columns together, one may conclude that the parasite encountered a favorable medium for growth in all three of the species concerned. In *F. diaphanus* there is a marked difference in the abundance of the Myxobolus as compared with either *F. majalis* or *F. heteroclitus*. In two cases of *F. majalis,* one is justified in assuming that there were large numbers of parasites, though they were not actually seen, because in one case the fish died of the disease and the slide preparation of its tissues was in some way lost; in the other case the extremely degenerate condition of the tissue justifies one in the expectation that no parasites will be found. Had the slide included muscle near the edge of the lesion, it is certain, on the basis of previous observations, that a large number of parasites would have been found.

One may conclude so far as this experiment goes that *F. diaphanus* is less favorable to the growth and multiplication of the Myxobolus. By reference to Columns 6 and 7, it is clear that, notwithstanding the smaller number of parasites, the disease is equally if not more destructive, having produced extensive necrotic sores and killed all specimens of *F. diaphanus* in three days. The unfortunate failure of the sea water at the end of nineteen hours prevented an interesting comparison of the endurance of the three species with reference to this parasite.

These observations prove beyond doubt that there is a succession of multiplicative cycles, and that large trophoblasts do not pass directly into the propagative condition. The propagative stages are distinctive and easily recognized both by their habit and staining qualities. It is now certain that some considerable multiplication in the multiplicative individuals involving several cycles must intervene before the propagative trophoblasts are produced.

The objection may be made that the culture utilized in the above-mentioned experiments was not pure, since one fish known as 1097.9 proved to be afflicted with both *Chloromyxum funduli* and *M. musculi.* It is necessary to admit that one could not with precision distinguish the trophoblasts of the Chloromyxum from those of the Myxobolus unless conditions happened to be very favorable. This is not the case, however, if either of these parasites are in the propagative cycle. In this case all the stages are distinctive for the two genera. There are besides this two very good reasons for believing that the fish from which these primary cultures were taken did not harbor Chloromyxum to the exclusion of Myxobolus: (1) The Fish 1097.9 is the second case

of *Chloromyxum funduli* which I have observed in the tissues of many hundred infected Fundulus; (2) no recognizable stages of *C. funduli* could be found in the material available in any of the other twelve fish mentioned above. One would hardly expect this particular combination of circumstances which would provide only one example of a parasite in the propagative cycle when they usually advance simultaneously from stage to stage, and at the same time that the initial infection be of rare occurrence, one which is encountered about one time in two hundred.

The inoculation experiments which follow are of a similar character to the above, and give support to and throw additional light upon some of the conclusions mentioned above. The purpose, however, was to aid in solving two questions which arise from the following circumstances. I have observed slight differences in the size of the myxospores from the gill and from the flesh of the Fundulus. In the gill I have encountered a range of variability in length from 13.4 to 12μ,

Figure 1 Figure 2 Figure 3

Fig. 1.—Cyst from gill filaments of Fundulus containing four myxospores of *M. musculi*. The cytoplasm around the myxospores is unstained. In this particular gill there were a number of these cysts.

Fig. 2.—Cyst from gill filaments of Fundulus containing a small number of myxospores of *M. musculi*. A conspicuous granular cyst plasm with definite outer wall characterizes this common type of encystment in the gill.

Fig. 3.—Cyst from filaments of Fundulus containing a large number of myxospores of *M. musculi* which have been assembled without any evidence of surrounding cyst plasm. There is, however, a definite limiting membrane. 68 by 67μ.

and in width from 10.4 to 6μ. For those seen in the flesh we have recorded elsewhere an average length for apparently mature myxospores of 14.3μ and an average thickness of 6.7μ. For obviously immature myxospores the dimensions average about 12 by 7.5μ. The size difference is therefore rendered invalid as an evidence of difference by the element of age. Another possible specific difference is suggested by the occurrence of myxospores both singly and in sporocysts of different sizes (Figs. 1, 2, and 3) in the gills, whereas in the flesh they

are usually isolated in our smear preparations. This difference can scarcely be due to the process of making smear preparations, because one should at least find the myxospores clustered if not occasionally in pseudocysts. It is very probable in view of what follows that the myxospores are either mechanically aggregated in the gills or if normally so related, they are mechanically distributed by the action of muscular contraction.

In order to finally settle this question of identity it was planned to introduce some of the myxospores of the gill, and if it so happened, some of their related trophic stages, into the body muscle. If the species were not identical, one would expect a marked difference in the pathological conditions and general habit of the parasite, if indeed it would grow at all. Some entire gill filaments of *F. heteroclitus,* 1098, which contained the myxospores of a Myxobolus in large clusters, singly and in sporocytes having four myxospores in each (Fig. 1), and large multiplicative or possibly propagative trophoblasts, were introduced under the integument of a *F. heteroclitus* 6.5 inches long. In four days the infected fish was dying. The mouth was gaping and there was an acute inflammation around the mouth and head. A large lesion had developed around the incision and the adjacent flesh under the unbroken swollen integument was a purulent mass. It was a typical myxosporidian wound. The muscle fibers of the fish were abundantly infected with numerous small multiplicative trophoblasts, many large trophoblasts and also large masses of multinucleated sporoblasts.

Unfortunately the water with which these fish were supplied was exposed to contamination by other infected fish. The head infection was doubtless due to direct contamination by handling or to the infected water. But I believe the flesh to have received its deep-seated and profound infection from the fragment of gill which was introduced.

The contaminated water made it necessary to repeat the experiment. As a number of *Cyprimodon variegatus* were available it was planned to test the possibility that *M. lintoni* and *M. musculi* are one and the same species (Hahn, 1913: 206). The gill filaments containing one or more large pseudocysts composed of apparently mature myxospores of the genus Myxobolus were removed from *F. majalis*. After carefully isolating a single filament it was introduced under the integument overlying the body muscle. The details of the experiment with summary of the observations will be found in Table 2.

It should first be noted that Fish 1103.1 died in less than a day, and thereupon in Column 8 the visible injury is found to be slight. The same condition prevails in 1103.3, but in 1103.2, 1103.4, 1103.7, and 1103.8 no reason can be given for the non-development of a typical lesion.

If one considers Column 8 it is impossible to deny that in some cases, at least, typical lesions do develop; but the evidence is not conclusive. The regular occurrence of one or more stages of the parasite in the flesh as indicated by Column 9 certainly forbids the conclusion that the myxobolus of the gill will not grow in the flesh. Allowing for the fact that one does not always happen to include in a smear prepartion Myxosporidia when present, it may be assumed that all the tissues reported in Column 9 contain multiplicative trophoblasts. No propagative stages were encountered. In those fish that lived forty-five hours and seven days were found large trophoblasts and stages which I have considered practically mature, i. e., schizonts. This fact harmonizes with the assumption that the transplanted myxospores have given rise to the new infection.

When compared with Columns 8 and 9 of Table 1, Columns 8 and 9 of Table 2 are not strikingly different, especially if one takes into consideration the period of development (twenty-two to forty-five hours), and a possibly longer time required for a myxospore to germinate. One must also consider the relative numbers of individual parasites represented in a bit of flesh containing hundreds of individuals and a bit of gill filament with only one or two pseudocysts like that in Figure 3. Obviously far more significance must be attributed to the presence of parasites at all, as indicated in Column 9, than is at first apparent. Considering the fragile nature and the relative size of myxospores which vary at different stages of development, and the difference in the nature of pseudocysts which may be either mechanical or due to too limited observations, I feel justified in taking the view that there is but one species of Myxobolus in Fundulus, and that it can be transplanted both by myxospores and trophic stages.

The case of the identity of *M. musculi* and *M. lintoni* is more perplexing. Since *M. musculi* grew readily (see Table 2) in *F. diaphanus* from fresh water, it might be supposed that it would grow more or less in the flesh of *C. variegatus*. If, on the other hand, the growth in *C. variegatus* had produced a typical tumor and the large type of myxospore had been recovered (Hahn, 1913: 206) we might find in the above observations evidence of the identity of the two species. It should be recalled that the *M. lintoni,* described by Linton (1891) and Hahn (1913), produced in all cases a very characteristic dermal tumor, which caused the death of the fish, according to Hahn, in a period of from two to three days. Such tumors are never encountered in the Fundulus, and nothing suggesting them was produced in the Cyprinodon of this experiment. On the other hand, *M. musculi* produces a typical ulcer in every way comparable to that in Fundulus.

It is worthy of note that, though the number of cases is small, there was an apparent difference between the number of parasites found in

F. heteroclitus and in either *F. majalis* or *C. variegatus*. This, together with the slight difference in the degree of development of the lesion, indicates that the parasite grows more readily in the muscle of *F. majalis* and *C. variegatus* than in *F. heteroclitus*. The number and maturity of the myxospores introduced must be taken into account. The conclusion is therefore not positive.

The above experiment, of which Table 2 is a summary, furnishes a minor contribution to the life-history of *M. musculi*. It would appear that if a pure myxospore culture were used in the inoculations, and if after seven days large schizonts are found in the second host, that not more than seven days is required for the parasite to pass from sporoplasm to schizont. Reference to Columns 6 and 9 of the table shows that such was the case in Fishes 1103.7 and 1103.8. But Fish 1103.5 had many large schizonts which must have developed in a forty-five-hour period. At least one cycle may therefore be completed in forty-five hours, and probably less, since the number of individual parasites in the twenty-two-hour cultures was far greater (1103.2) than the number of myxospores introduced. This conclusion is not absolutely certain inasmuch as a gill filament containing a pseudocyst might also contain other stages unseen, but it is very improbable if the myxospores are ripe. If trophoblasts were present, they were not numerous and the time relations above recorded would then apply to the period of a cycle starting with a multiplicative spore rather than a myxospore.

The ease with which one can introduce either multiplicative spores and trophoblasts or myxospores and probably propagative trophoblasts into the tissues of a healthy fish provides a very plausible explanation of the way by which fish whose integument has been broken may pick up the Myxobolus from the water. Thus, though commonly in the gill and head region where it is comparatively harmless, it comes to react on the body muscle where it is oftentimes fatal. Rough handling and close confinement in aquaria tend to provide the ideal conditions for the infection of the muscle.

A final solution of a question which confronted the writer during the first stages of his investigations of *M. musculi* (Hahn 1913: 199), namely the possible causative relation of certain bacilli to the pathological changes in *F. musculi,* is found in the inoculation experiments. When one can produce at will the typical condition by the use of Myxobolus myxospores but fails to get it by laceration, one may conclude that the bacteria are purely secondary. Moreover, numerous preparations show that the vanguard of the infection is always a tissue comparatively free from bacteria. I am not prepared to say that bacteria do not poison and kill the host as secondary agents. They are probably saprophytic and the primary Myxosporidian parasite prepares the way for them.

SUMMARY OF RESULTS OBTAINED IN INOCULATION EXPERIMENTS

1. M. musculi is communicable in all stages of its life-history.

2. Many multiplicative cycles are repeated before *M. musculi* passes into its propagative cycle.

3. The Myxobolus which is very common in the gills, where it is seldom destructive, is identical with that which occurs in the flesh.

4. Infection of lesions in the integument takes place upon the entrance of any stage of *M. musculi* from contaminated water. The water is presumably contaminated from the gills.

5. Transplanted *M. musculi* may continue for some time in the same cycle in the new host. Or they may pass into the next cycle soon after the transfer.

6. Myxospores germinate when transplanted to another fish and produce schizonts in considerable less than one day.

7. The multiplicative cycle requires less than one day and probably takes place many times in this period.

8. The propagative cycle may be reached in 48 hours.

9. Recovery from infection with *M. musculi* is possible if the body muscle is not involved and if the fish are fed and supplied with oxygen. Eye muscles and possibly other parts of the head are also vital.

10. Recovery is probably possible if the infection occurs when the parasite is in or near the end of the propagative cycle even when the body muscle is involved.

11. Progress of the disease is slow in the integument.

12. The parasite almost invariably migrates from hypertrophied tissues.

13. Passage from stage to stage is approximately simultaneous.

14. *Fundulus majalis, heteroclitus,* and *diaphanus* and *Cyprinodon variegatus* are culture media for *M. musculi.. C. variegatus* is a little less favorable for its growth but is perhaps less immune to the toxic products evolved in this particular kind of a lesion.

15. The Myxobolus from the gill of Fundulus is identical to that which is common in the flesh.

16. There is no valid reason as yet to consider *M. musculi* and *M. lintoni* of the *Cyprinodon variegatus* as one and the same species. On the other hand the bulk of the evidence favors the opposite view.

17. The associated bacteria are either purely saprophytic or secondary parasites which gain entrance from the water and find the natural resistance of the tissues lowered by the Myxobolus. The latter invade the normal tissue and leave the atrophied tissues to the bacteria.

Observations upon the multiplicative stages of *M. musculi* will follow in an early number of THE JOURNAL OF PARASITOLOGY.

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